(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: **0 537 654 A2**

(12) EUROPEAN PATENT APPLICATION

(21) Application number: **92117373.8**

(22) Date of filing: **12.10.92**

(51) Int. Cl.5: **C12P 21/08**, C12N 5/26, A61K 39/395, G01N 33/577, G01N 33/574

The microorganism has been deposited with DSM under number DSM ACC2023

(30) Priority: **18.10.91 EP 91810807**

(43) Date of publication of application: **21.04.93 Bulletin 93/16**

(84) Designated Contracting States: **AT BE CH DE DK ES FR GB GR IE IT LI LU MC NL PT SE**

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(54) Anti-alpha6-integrin-antibodies.

(57) The present invention is directed to a monoclonal antibody or a fragment thereof which is characterized by its ability to bind to mammalian α6-integrin, preferentially human α6-integrin, and to inhibit metastasis, to a hybridoma cell line secreting such an antibody, a process for the preparation of such a hybridoma cell line and the antibody, pharmaceutical compositions containing such an antibody or a fragment thereof and the use of the antibody or a fragment thereof for the treatment of illnesses, especially cancer.

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Adhesion of invasive cancer cells on vascular endothelium is a critical first and selective step in metastasis. During the last years a large number of different adhesion molecules have been discovered to be involved in cell migration and homing mechanisms of hemopoietic cells. Transformed tumor cells can obviously abuse such mechanisms in an uncontrolled way leading to metastasis in tissues different from their origin.

Now it has been surprisingly found on the basis of the specific ability of the monoclonal antibodies of the present invention to inhibit metastasis that a further molecule, namely the antigen recognized by these monoclonal antibodies, which is known as α6-integrin [for a review see Hemler in Annu. Rev. Immunol. 8, 365-400 (1990)], can be also considered as a and probably the most important endothelial adhesion molecule involved in metastasis.

Three other antibodies directed against the α6 integrin chain have been described in the literature whereby the methological approaches for their production were totally different. Antibody GoH3 was produced by immunizing rats with blood platelets, it was selected because it recognized the platelet protein complex Ic-IIa subsequently defined as α6/β1 integrin [Sonnenberg et al., J. Biol. Chem. 262, 10376-10383 (1987); Hemler et al., J. Biol. Chem. 263, 7660-7665 (1988)]. Antibody 135-13C was prepared in rats against purified tumor associated proteins TSP-180, now described as α6/β4 integrin [Kennel et al., Cancer Res. 41, 3465-3470 (1981); Kennel et al., J. Biol. Chem. 264, 15515-15521 (1989)]. Finally, antibody GB36 was raised in mice against microvilli preparations of human placenta. The antibody recognized cell surface proteins on human carcinoma cells [Hsi et al., Placenta 8, 209-217 (1987)]. It has been suggested that the recognized protein (α6/β1 integrin) may play a role in maitenance of cell polarity, however no functional assays have been performed.

Carcinoma, melanoma and endothelial cells can adhere to the extra-cellular matrix molecule laminin. Several integrin complexes including α1/β1, α2/β1 and α6/β1 participate in mediating this binding. One of them, α6/β1, seemed to react monospecific for laminin and the antibody GoH3 can block cell-laminin interaction and it defined α6 integrin as a receptor for the E8 fragment obtained by an elastase digest (Sonnenberg et al., 1990). Antibody 135-13C also had an effect on cell binding to laminin but to a lower extent.

The antibody of the present invention does not interfere with the binding of cells to laminin but blocks cell-cell interaction of melanoma cells with vascular endothelial cells. This finding strongly suggests that the antibody of the present invention recognizes a so far unknown binding domain on the α6 integrin chain clearly different from the binding sites of GoH3, 135-13C or GB36. This can be confirmed furthermore by the fact that GoH3 cross-reacted with skin homing lymphocytes in sheep whereas the antibody of the present invention does not.

Accordingly it is an object of the present invention to provide a monoclonal antibody or a functional derivative thereof, especially a Fab-fragment, which is characterized by its binding to mammalian, e.g. mouse or preferentially human α6-integrin [anti-α6i-mAb] and which inhibits metastasis of transformed cells, especially by inhibiting the interaction of the transformed cells with the vascular endothelium. Such transformed cells are, generally speaking, those which on the one side are characterized by carrying α6-integrins as surface molecules as e.g. melanomas, carcinomas, T cell lymphomas or sarcomas, with melanomas and carcinomas, especially melanomas being preferred and on the other side those which carry a so far undetermined ligand of α6-integrins as a surface molecule.

It is furthermore an object of the present invention to provide hybridoma cell lines secreting such monoclonal antibodies and the monoclonal antibodies secreted by such cell lines. A hybridoma cell line "F3C34" obtained in accordance with the teaching of the present invention has been deposited at the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSM). It is furthermore an object of the present invention to provide such monoclonal antibodies or fragments thereof as therapeutic agents, especially for the treatment of cancer by the inhibition of metastasis.

Antibodies binding to α6-integrins can be prepared by using short peptides with amino acid sequences derived from the known amino acid sequence of human α6-integrin [Hogervorst et al., Eurp. J. Biochem. 199, 425-433 (1991)] as starting antigen. Such peptides can be prepared by methods known in the art of chemical peptide and protein synthesis, e.g. by partial or total liquid or solid phase synthesis as described e.g. by Gross and Meyenhofer in "The Peptides" Vols. 1-9, Academic Press, Inc., Harcourt Brace Jovanovich, Publs., San Diego (1979-1987) or by Fields and Nobel, Int. J. Pept. Prot. Res. 35, 161-214 (1990).

However, antibodies raised in such a way do not necessarily react with the native α6-integrins nor show the specific properties of those of the present invention. Therefore antibodies of the present invention can be prepared starting from α6-integrin positive cells, e.g. endothelial cells or transformed cells of endothelial origin, whereby an eEnd2-cell line is specifically preferred.

By injection of such an antigen into a non-human mammal, e.g. mouse, rabbit, rat or sheep, polyclonal antibodies can be obtained by methods known in the art from the serum. Monoclonal antibodies can be prepared by recovering antibody producing cells from such an immunized animal and immortilizing said cells obtained in conventional fashion like fusion with myeloma cells e.g. PAI mouse myeloma cells, SP2/0- or SP2/0-Ag14-cells [ATCC No. CRL 1581; ATCC No. CRL 8287] [for a general guideline for producing antibodies see e.g. "Antibodies-A Laboratory Manual" edt. by Harlow, E. and Lane, D., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, 1988] or as described in detail in Example I.

Supernatants of such hybridoma cultures have then to be screened for those secreting antibodies of the present invention by an assay determining the metastasis inhibiting properties of the antibodies of the present invention, e.g. an assay as described in detail in Example I. It is therefore also an object of the present invention to provide a process for the preparation of anti-α6i-mAbs and fragments thereof according to methods known in the art whereby hybridoma supernatants obtained are screened by the ability of the antibodies contained in such supernatants to inhibit metastasis and compounds obtained by such a process.

Anti-α6i-mAbs can be purified from hybridoma supernatants by conventional chromatographic procedures like, for example, by ion-exchange-chromatography, affinity chromatography on protein G, antiimmunoglobulin-antibodies or the antigen or a part thereof bound to a solid support, HPLC or the like.

For the production of large quantities of anti-α6i-mAbs in accordance with methods well-known in the art hybridomas secreting the desired antibody can be injected intraperitoneally into mice which have been pretreated with for example pristane before injection. Up to around 100 mg of a monoclonal antibody can be produced by such ascites tumors in one mice. Antibodies can be purified for example from ascites fluid produced by such tumors using methods as stated above.

Antibodies of the present invention can be characterized according to their subclass by known methods, such as e.g. Ouchterlony immunodiffusion. The antibody secreted by hybridoma cell line "F3C34" is of the IgG2a subtype. Furthermore antibodies of the present invention can be characterized by their ability of inhibiting metastasis of transformed cells, especially the interaction of the transformed cells with the vascular endothelium. Such activity can be determined by assays known in the art, e.g. in vitro by an assay as described e.g. in Example I or in vivo by an assay as described e.g. in Example II wherein any transformed cell line carrying the α6-integrin as a surface molecule, e.g. melanomas as e.g. B16 cells [Vollmers et al., Cell 40, 547-557 (1985)], carcinomas, as e.g. KLN205 [ATCC CRL 1453] or CMT-93 [ATCC CCL 223] or MM45T [ATCC CRL 6420], T cell lymphomas, as e.g. BW 5147 [ATCC TIB 48] or sarcomas, as e.g. MM46T [ATCC CRL 6420] can be used in accordance with a suitable tissue material to which such cells and the antibodies of the present invention bind in case of the in vitro assay and type of animals in which such transformed cell lines lead to metastasis and to which the antibodies of the present invention crossreact. Inhibition of the interaction of such transformed cell lines with the vascular endothelium can be determined by assays known in the art, e.g. by an in vitro adhesion assay on monolayers of endothelial cells or cells of endothelial origin or as described in Example I and shown in Fig. 2 whereby arrows clearly point to the position of the transformed cells binding to the vascular endothelium. Finally antibodies of the present invention can be characterized by blocking cell migration in an assay as described in Example V or by not influencing cell growth in an assay as described in Example VI.

Furthermore antibodies of the present invention cross-react with human α6-integrin as demonstrated, e.g. by the detection of α6-integrin on the apical surface of human endothelial cells (see e.g. Example III) which correlates with in vivo staining of mouse α6-integrin on the apical surface of mouse blood vessel endothelium (see Example IV).

Anti-α6I-mAbs can be modified for various uses as known in the art or fragments thereof can be generated as described e.g. in Example II which still show binding of the antigen (Harlow & Lane s.a.). Fragments can be generated, for example, by enzymatic digestion of antibodies with papain, pepsin or the like. In addition the antibodies of the present invention can be modified e.g. by the addition of a polyethylenglycol subunit as known in the art and described e.g. in U.S. Patent 4.179.337 or coupled, for example, to a fluorescent dye, a colour producing substance, like an enzyme [enzyme linked immunosorbent assay (ELISA)] or a radioactive substance [radioimmunoassay (RIA)] in accordance with methods well-known in the art and used in such assay systems as known in the art. Furthermore such antibodies can be "humanized" according to methods known in the art and disclosed for example for an antibody specific for a subunit of the human interleukin 2 receptor in International Patent Application Publication No. WO 90/7861. Accordingly functional derivatives as described above are also an object of the present invention.

Monoclonal antibodies of the present invention may be used as therapeutic agents especially in the treatment of cancer e.g. by inhibiting metastasis especially in the case of secondary metastasis during surgery.

The antibodies can be used, if desired in combination with other pharmaceutically active substances, preferentially monoclonal antibodies or peptides against different adhesion molecules, all to be present on vascular endothelium or the metastatic cell, with conventionally used pharmaceutically acceptable solid or liquid carrier materials. Dosage and dose rates may be choosen in analogy to dosage and dose rates of currently used antibodies in clinical treatment of various diseases.

Accordingly it is also an object of the present invention to provide anti-α6i-mAbs which can be clinically used. Furthermore it is an object of the present invention to provide a pharmaceutical composition which contains one or more anti-α6i-mAbs or fragments thereof, if desired, in combination with additional pharmaceutically active substances and/or non-toxic, inert, therapeutically compatible carrier materials. The preparation of such pharmaceutical compositions can be achieved in accordance with methodology a man skilled in the art is familiar with.

Furthermore monoclonal antibodies of the present invention can be used as a marker in the diagnosis of illnesses, especially cancer. It is well-known in the art that for such purposes the monoclonal antibodies can be coupled, for example, to a fluorescent dye, a colour producing substance, like an enzyme [enzyme linked immunosorbent assay (ELISA)] or a radioactive substance [radioimmunoassay (RIA)] in accordance with methods well-known in the art and used in such assay systems as known in the art as described, e.g. in Marlow & Lane (s.a).

The following Examples are illustrating the invention without limiting it.

Example I

Preparation of anti-α6i-mAbs

Confluent endothelial cells (eEnd2-cell line) from a 150 cm^2 culture flask were irradiated with 10,000 rad and harvested with cell scrapers (Costar Data Packaging). These cells were then washed with Dulbecco's phosphate buffered saline (DPBS) (Gibco BRL), mixed 1:1 with complete Freund's adjuvant for a final volume of 300 µl, and injected subcutaneously into the dorsal surface of the hind foot of a 2-mouth-old PVG rat [BRL, Füllinsdorf, CH]. Injections with cells in DPBS only were repeated after 7 and 14 days. At day 17, the draining popliteal lymph node was dissected from the rat. The tissue was enzymatically digested using the following enzyme stock solutions: 150 mg/ml protease type IX (P-6141; Sigma Chemical Co., Buchs, CH); 8 mg/ml collagenase CLS 4 (Worthington Biochemical Corp., Freehold, NJ, USA); 10 mg/ml DNAse I (Sigma Chemical Co.). The enzyme solutions were mixed to a final volume of 2 ml (0.5 ml Collagenase, 0.1 ml Protease, 0.1 ml DNAse, 1.3 ml IMDM [Iscoves modified MEM; Gibco BRL, Gaithersburg, MD, USA]. A lymph node was opened by two slight crosscuts using a 25-gauge needle. Stroma were then digested at 37 °C for two 30-min periods each with 1 ml enzyme cocktail. The cells were then carefully released into IMDM with Pasteur pipettes, washed in 50 ml IMDM, and counted. One part lymph node cells was then mixed with five parts mouse Sp2/0 myeloma cells [ATCC CRL 1581], centrifuged, and fused with PEG 4000 (E. Merck, Darmstadt, FRG) as described in Harlow and Lane (1988). The cells were then plated (100 µl) into conditioned medium in microtiter plates (96 wells; Costar Data Packaging) at a density of 5×10^4 cells/well in IMDM selection medium containing HAT (Gibco BRL), 10% fetal calf serum (FCS) (Boehringer Mannheim GmbH, Mannheim, FRG), 50 µM β-mercaptoethanol, penicillin/streptomycin, and glutamine. Conditioned medium was produced by culturing 10^4 PVG rat thymocytes (100 µl/well) in selection medium for three days before fusion.

Screening of hybridoma supernatants for metastasis inhibiting mAbs was based on blocking of B16/129 melanoma cell binding to frozen sections of mouse lung or liver tissue.

In situ binding was performed on freshly prepared frozen sections from adult mouse tissue [Woodruff et al., Annu. Rev. Immunol. 5, 201-222 (1987)]. Organs were embedded and frozen in Tissue-Tek, O.C.T. Compound (Miles Inc., Elkhart, IN, USA), sectioned to 5 µm thickness, and mounted onto glass slides. The sections were outlined by a PapPen (SCI Science Services, Munich, FRG) and immediately placed into DPBS containing 1% bovine serum albumin (BSA) for at least 10 min. Slides were dried around the PapPen marked area and the tissue section then loaded with 10^5 B16 melanoma cells in 200 µl DPBS containing 20% hybridoma supernatant of cultures prepared as described above a control antibody (rat IgG from Jackson Immuno Research Lab). Binding was allowed to occur for 40 min at 8 °C on a mini-shaker (Kühner, Basel, CH) at 50 rpm. Slides were then placed vertically into DPBS containing 0.5% glutaraldehyde and 2% formaldehyde, where non-bound cells were allowed to fall off. After 20 min. of fixation at room temperature and counterstaining in 0.25% thionineacetate in 20% ethanol, B16 cells bound to the tissue section under study were counted using a Zeiss Axiophot light microscope. Results are given in Figure 1 with respect to lung and liver tissue sections and additional tissue sections (kidney, thymus, spleen and heart) prepared in

the same way as described above for lung and liver whereby left colums give mean values of bound B16 cell to tissues as indicated in the absence of anti-α6i-mAbs and right columns in the presence of anti-α6i-mAb containing hybridoma supernatants. Figure 2 shows lung tissue sections incubated with control supernatants (a) or anti-α6i-mAb containing hybridoma supernatants (b) and liver tissue sections incubated with control supernatants (c) or anti-α6i-mAb containing hybridoma supernatants (d).

Immunprecipitation with antibodies prepared as described above and partial N-terminal sequenceing of the precipitated antigen showed that the antibody is directed to mouse α6 integrin since the first 15 amino acids sequenced are besides position 14 (serine instead of tyrosine) identical to the sequence of human α6 integrin.

Example II

In vivo characterisation of anti-α6i-mAbs

Thawed B16-129 cells [Vollmers et al. (1985)], a subline from B16-F10 mouse melanoma cells (passage 3) were passeged maximally 2-3 times in DMEM (Gibco, BRL, Paisley, UK) containing 10% fetal calf serum (FCS) (Boehringer Mannheim GmbH, Mannheim, FRG) quickly trypsinized, washed with complete medium and resuspended in PBS. Intraveneous injection of $1.5x10^5$ cells resulted in 300-500 lesions per lung after 10 days, injection of $7.5x10^4$ resulted in 30-50.

For characterisation of anti-α6i-mAbs in vivo B16-129 cells were injected i.v. into mice together with control rat-mAb or anti-α6i-mAbs or Fab fragments thereof. Lung B16-129 colonies were counted after 10 days. Results are given in Tables I and II.

With respect to Table I ten mice per group were injected with $7.5x10^4$ cells each. With respect to Table II mice were injected with $1.5x10^5$ B16-129 cells: (a) injection of cells was made simoultaneous with the antibodies, (b) antibodies were injected 24 hours before the cells and (c) melanoma cells were incubated 60 min. with antibodies, washed and then injected into animals. The table represents one out of three experiments performed, 5 mice were injected per group.

Table I

	Control	500 µg anti-α6i-mAb/mouse	250 µg Fab of anti-α6i-mAb/ mouse	500 µg Fab of anti-α6i-mAb/ mouse
Lung lesions per mouse	37 (5-113)	1 (0-4)	0	0

Table II

Type of treatment	Control	Fab of anti-α6i-mAb	Reduction of lung lesions
Simultaneous injection (a)	610 (321-993)	147 (3-481)	86%
Pretreatment of mice (b)	742 (321-1025)	474 (45-1008)	36%
Pretreatment of B16-129 melanoma (c)	465 (208-563)	224 (145-253)	62%

Anli-α6i-mAbs were purified from hybridoma supernatant using protein G affinity columns (Pharmacia, Uppsala, Sweden). Fab fragments thereof were prepared using the kit "AvidChrom" (BioProbe International, Tustin, CA, USA). As control normal rat IgG (Jackson Immuno Research Laboratories, West Grove, PA, USA) was used.

Example III

Binding of anti-α6i-mAbs to human endothelial cells

Binding of anti-α6i-mAbs on the apical surface of human endothelial cells was shown in the following manner. HUV-EC-C-cells [ATCC CRL 1730] were grown to confluency as known in the art, stabilized by formaldehyd fixation and incubated with a graded dilution of a 100 µg/ml stock solution anti-α6i-mAbs. Surface bound antibody was detected by biotinylated mouse anti-rat antibody (Jackson Immuno Research,

West Grove, PA, USA) followed by streptavidin coupled to peroxidase and 2,2'-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid), ABTS (Sigma), as a substrate readable at 405 nm. Results are shown in Figure 3.

Example IV

Vascular surface staining in vivo

1 mg of protein G-sepharose purified anti-α6i-mAbs was injected into the tail vein of C57B1/6 mice [IFFAC-CREDO, Lyon, F]. Animals were sacrificed 4 hrs after injection, the tissues embedded in Tissue-Tek (Miles Inc, Elkart, IN 46515, USA) sectioned on a cryostat and prepared for immunofluoresence staining using FITC conjugated goat anti-rat IgG antibody (Jackson Immuno Research, West Grove, PA, USA). Fig. 4(a) shows a phase contrast image and Fig. 4(b) an immunofluoresence staining of lung. Fig. 4(c) shows a phase contrast image and Fig. 4(d) shows a immunofluorescence stain of liver. Injection of 1 mg rat IgG as a control showed no signal.

Example V

Blocking of cell migration

B16-129 melanoma cells were plated to confluency in 12 well culture cluster dishes (Costar) in medium containing 0.3, 3 or 10% fetal calf serum. The monolayer was then wounded by scratching the dish using blue pipette tips (Gibco BRL, Gaithersburg MD, USA), this leaved a sharply separated region without cells. Cell migration into this region was subsequently observed after further 24 hours culturing. There was no influence on cell migration when the cells were grown in either 3 or 10% serum (not shown), however in 0.3% serum anti-α6i-mAb blocked B16-129 cell migration dramatically. Figure 5 (a) shows the migration of cells in 0.3% serum towards the center of the wounded region in the presence of 30μg/ml control rat IgG and (b) in 30μg/ml anti-α6i-mAb.

Example VI

Influence on cell growth

B16-129 melanoma cells were plated in 24 well culture cluster dishes (3524, Costar, Cambridge, MA, USA) at a density of 10^4 cells per well in medium containing either 0.3, 3 or 10% fetal calf serum in the presence of 30 μg/ml anti-α6i-mAb (A) or as a control in the presence of 30 μg/ml of a rat IgG-mAb (B). Over 4 days the cells from two wells per condition were trypsinized and counted. The cells grew readily in 10 and 3% serum but they were quiescent in 0.3% serum over the 4 days tested. Under neither of these conditions anti-α6i-mAb had any effect on cell growth. Results are given in Figure 6 whereby filled boxes refer to 0.3% of fetal calf serum and (B), filled circles refer to 0.3% and (A), open boxes refer to 3% of serum and (B), open triangels refer to 3 % serum and (A), filled triangels refer to 10% of serum and (B) and open circles refer to 10% of serum and (A).

Claims

1. A monoclonal antibody or a functional derivative thereof which is characterized by its ability to bind to mammalian α6-integrin and to inhibit metastasis.

2. A monoclonal antibody or a functional derivative thereof as claimed in claim 1 whereby the mammalian α6-integrin is human α6-integrin.

3. A hybridoma cell line secreting a monoclonal antibody as claimed in claim 1 or 2.

4. A compound as claimed in claim 1 or 2 as a therapeutic agent, especially for the treatment of cancer, or a diagnostic marker.

5. A process for the preparation of a compound as claimed in claim 1 or 2 characterized in that α6-integrin positive cells are injected as starting antigen into a non-human mammal, antibody producing

cells are recovered from such immunized animal and immortalized in conventional fashion and the resulting hybridoma cell lines are screened by an assay determining inhibition of metastasis, the secreted antibody is isolated from the supernatant of the corresponding hybridoma cell line and if desired, cleaved enzymatically or chemically.

6. A pharmaceutical composition which contains one or more compounds as claimed in claim 1 or 2, if desired, in combination with additional pharmaceutically active substances and non-toxic, inert, therapeutically compatible carrier materials.

7. The use of a compound as claimed in claim 1 or 2 for the treatment of illnesses, especially for the treatment of cancer.

8. The use of a compound as claimed in claim 1 or 2 for the diagnosis of illnesses, especially cancer.

Fig. 1

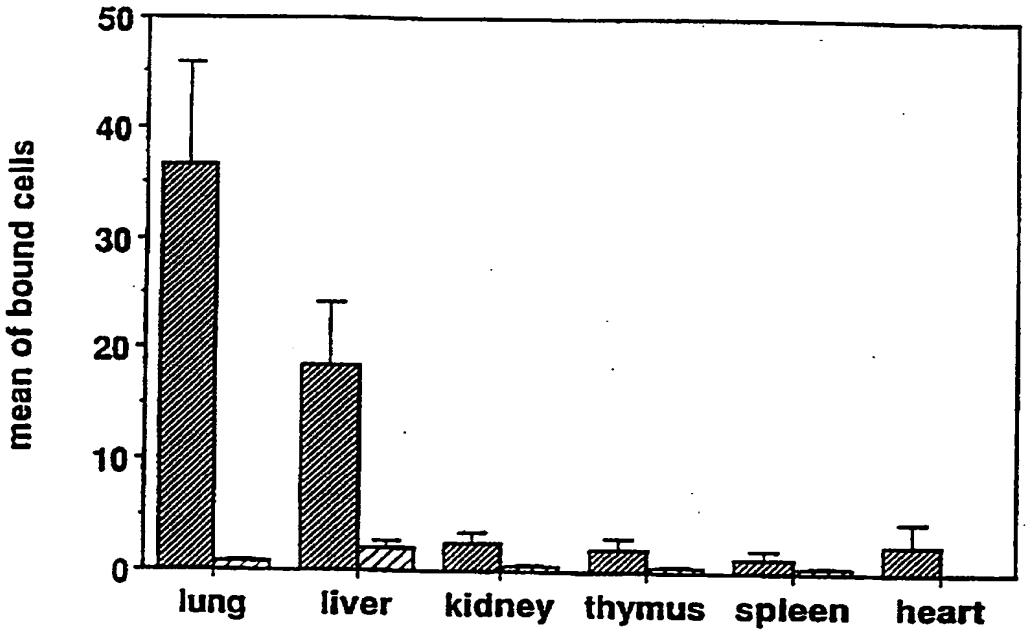


Fig. 2

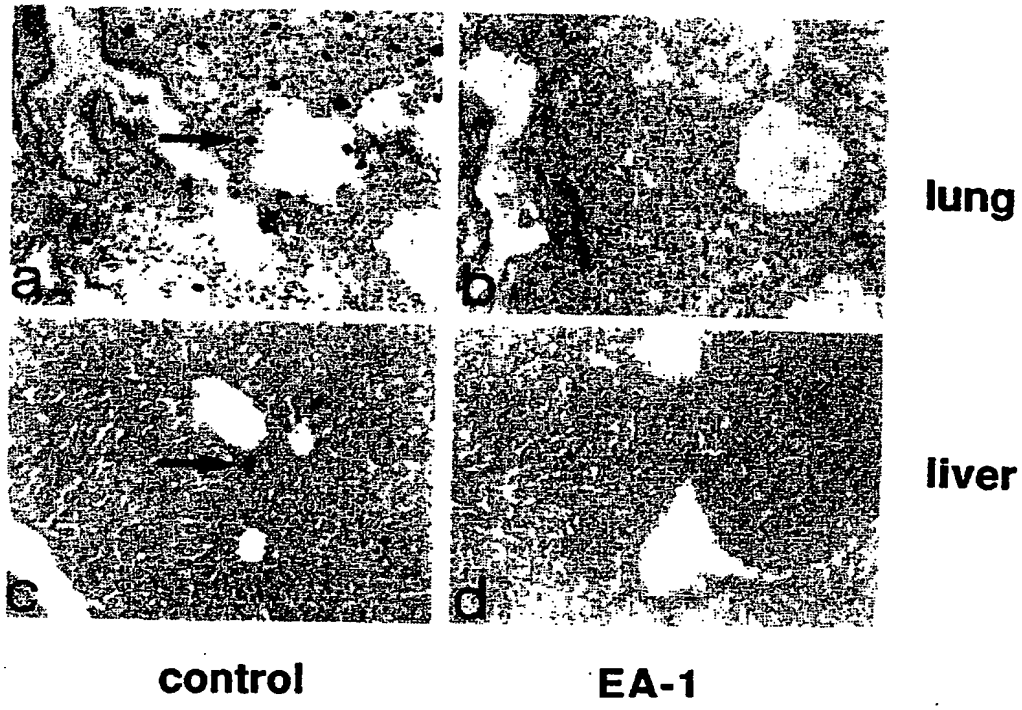


Fig. 3

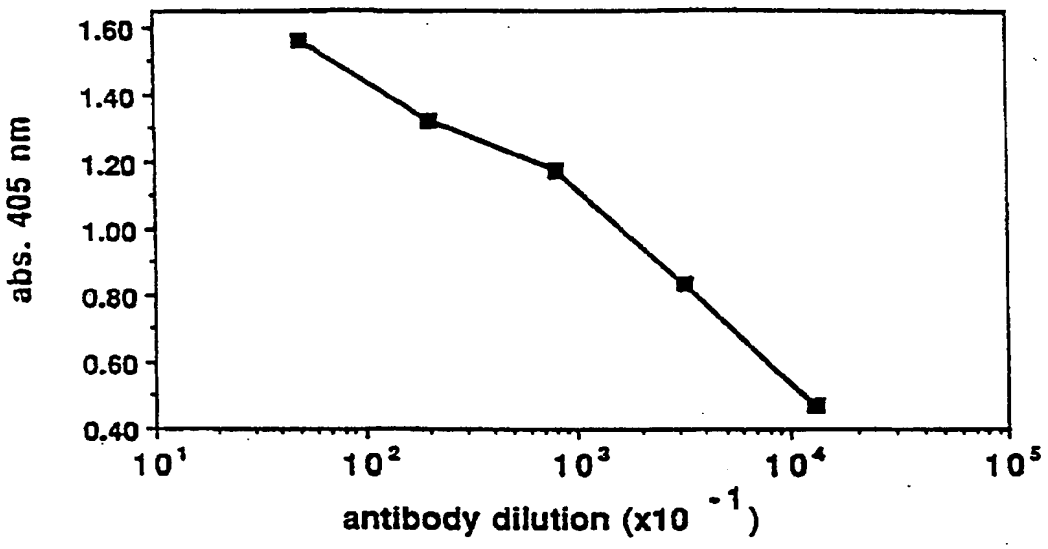


Fig. 4

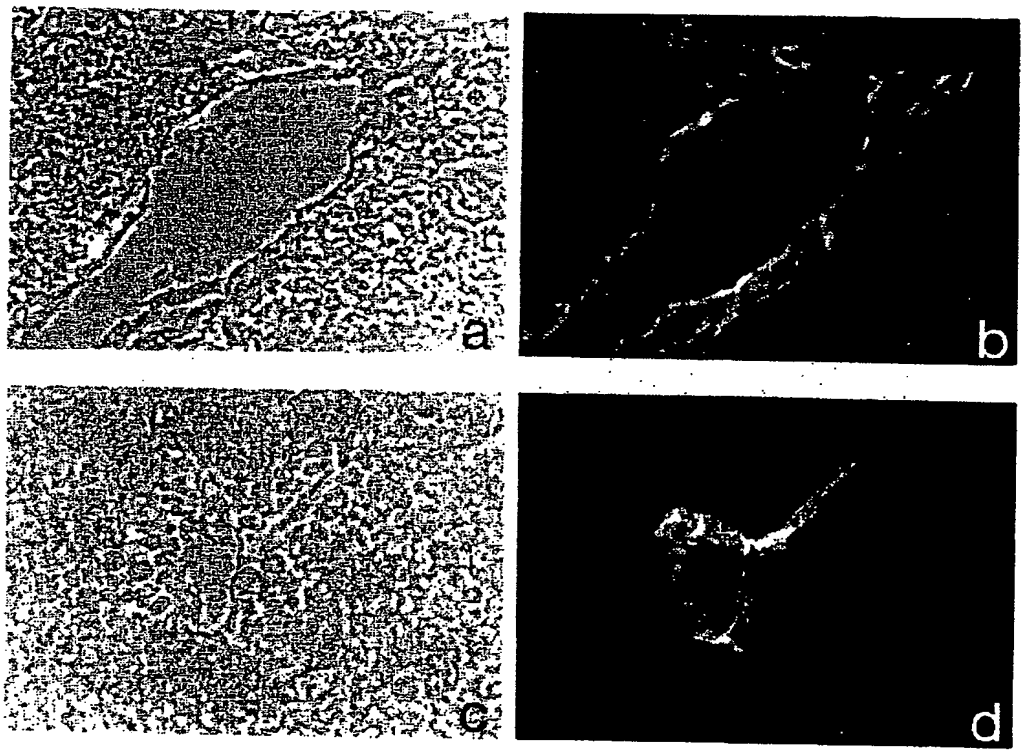
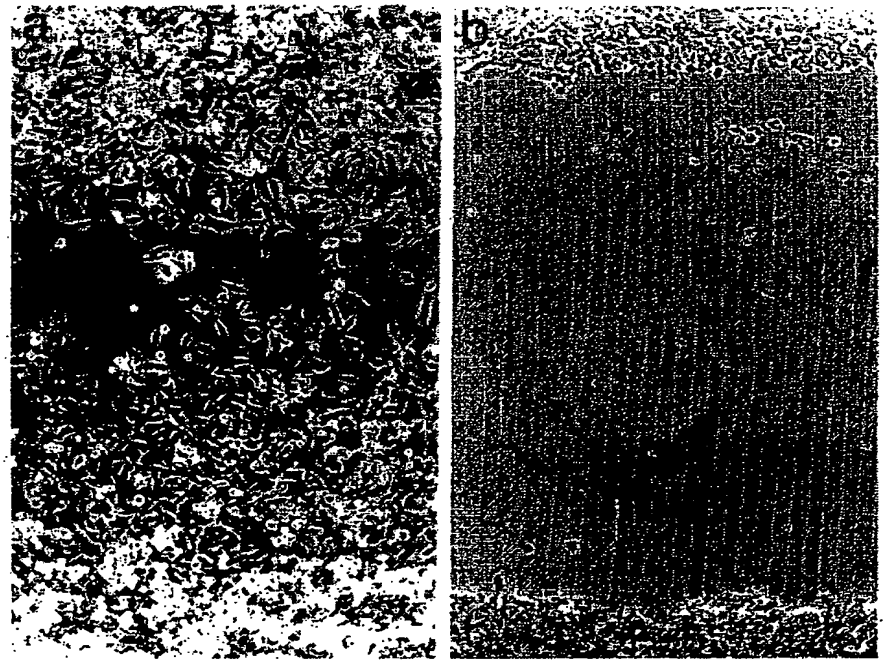


Fig.5



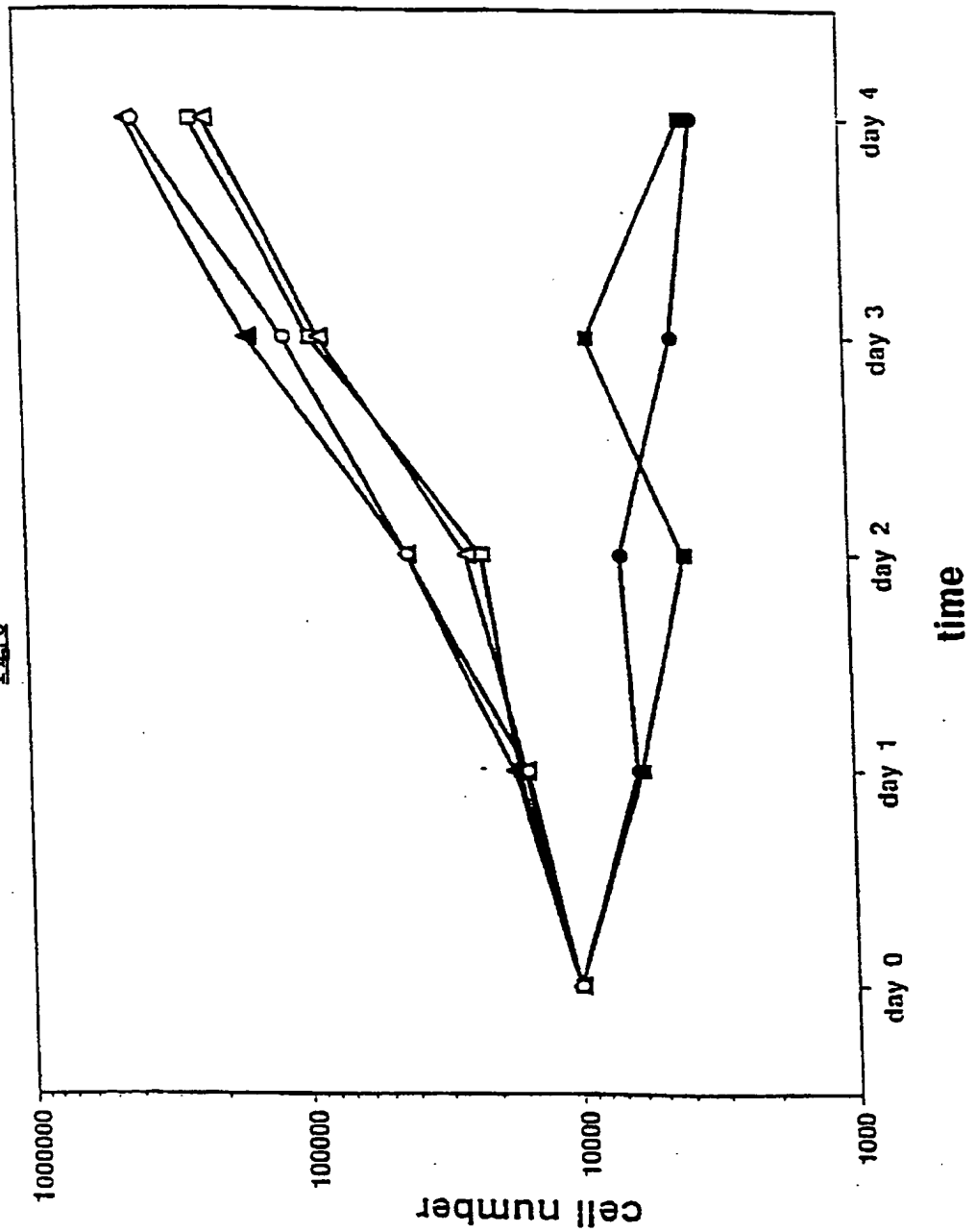


Fig 6

(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

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(43) Date of publication of application: **21.04.93 Bulletin 93/16**

(84) Designated Contracting States: **AT BE CH DE DK ES FR GB GR IE IT LI LU MC NL PT SE**

(88) Date of deferred publication of the search report: **29.12.93 Bulletin 93/52**

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European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

which under Rule 45 of the European Patent Convention shall be considered, for the purposes of subsequent proceedings, as the European search report

Application Number EP 92 11 7373

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl. 5)
X	ANTICANCER RESEARCH vol. 10, no. 6, November 1990, ATHENS, GREECE pages 1587 - 1598 D. PEROTTI ET AL. 'Metastatic phenotype: Growth factor dependence and integrin expression.' * abstract * ---	1-8	C12P21/08 C12N5/26 A61K39/395 G01N33/577 G01N33/574
X	CANCER RESEARCH vol. 50, no. 3, 1 February 1990, PHILADELPHIA PA, USA pages 728 - 734 D. RAMOS ET AL. 'Analysis of integrin receptors for laminin and type IV collagen on metastatic B16 melanoma cells.' * the whole document * --- -/--	1-8	

TECHNICAL FIELDS SEARCHED (Int. Cl. 5)

C12P
C12N
A61K
G01N

INCOMPLETE SEARCH

The Search Division considers that the present European patent application does not comply with the provisions of the European Patent Convention to such an extent that it is not possible to carry out a meaningful search into the state of the art on the basis of some of the claims

Claims searched completely :

Claims searched incompletely :

Claims not searched :

Reason for the limitation of the search:

see sheet C

Place of search	Date of completion of the search	Examiner
THE HAGUE	11 OCTOBER 1993	NOOIJ F.J.M.

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
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T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P0407)

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int. Cl. 5)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
X	AMERICAN JOURNAL OF PATHOLOGY vol. 138, no. 3, March 1991, HAGERSTOWN MD, USA pages 741 - 750 K. KORETZ ET AL. 'Expression of VLA-alpha2, VLA-alpha6, and VLA-beta1 chains in normal mucosa and adenomas of the colon, and in the colon carcinomas and their liver metastases.' * abstract * * page 748, left column, line 35 - line 45 * ----	1-8	TECHNICAL FIELDS SEARCHED (Int. Cl. 5)

EPO FORM 1503 03.82 (P04E10)

Remark: Although claim 7 is directed to a method of treatment of the human/animal body (Art. 52(4) EPC), the search has been carried out and based on the alleged effects of the compound/composition.
Although claim 8 (partially, as far as an in-vivo diagnosis is concerned) is directed to a diagnostic method practized on the human/animal body (Art. 52(4) EPC), the search has been carried out and based on the alleged effects of the compound/composition.